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Bed bug cytogenetics: karyotype, sex chromosome system, FISH mapping of *18S rDNA*, and male meiosis in *Cimex lectularius* Linnaeus, 1758 (Heteroptera: Cimicidae)

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Abstract. Bugs (Insecta: Heteroptera) are frequently used as examples of unusual cytogenetic characters, and the family Cimicidae is one of most interest in this respect. We have performed a cytogenetic study of the common bed bug *Cimex lectularius* Linnaeus, 1758 using both classical (Schiff-Giemsa and $AgNO_3$-staining) and molecular cytogenetic techniques (base-specific DAPI/CMA_3 fluorochromes and FISH with an *18S rDNA* probe). Males originated from a wild population of *C. lectularius* were found to have $2n = 26 + X_1X_2Y$, holokinetic chromosomes, *18S rRNA* genes located on the X_1 and Y chromosomes; achiasmate male meiosis of a collochore type; MI and MII plates nonradial and radial respectively.

Key words: holokinetic chromosomes, karyotype, multiple sex chromosomes, achiasmate collochore meiosis, FISH with an *18S rDNA* probe, *Cimex lectularius*.

Introduction

The bed bug genus *Cimex* Linnaeus, 1758 is a relatively small group of highly specialized hematophagous ectoparasites, with 17 species distributed primarily across the Holarctic and associated with humans, bats, and birds (Schuh, Slater, 1995; Simov et al., 2006). Till now, the chromosomal complement and different aspects of chromosome behavior during male meiosis have been studied in 14 *Cimex* species (Ueshima, 1963, 1966; Grozeva, Nokkala, 2002; for other references see Ueshima, 1979), including the common bed bug *C. lectularius* Linnaeus 1758 (Slack, 1938, 1939a, b; Darlington, 1939; Ueshima, 1966). As is the case with other Heteroptera, *C. lectularius* displays holokinetic chromosomes, i.e. chromosomes having, instead of localized centromere, a kinetochore plate spread along their whole or almost whole length. Among several peculiarities of *Cimex* cytogenetics, multiple sex chromosome systems are the most conspicuous. Although, within Cimicidae, multiple sex chromosomes are not unique to the genus *Cimex* (some species in at least nine other Cimicidae genera have multiple X chromosomes; see Ueshima, 1979), the number of X is, by far, greatest in *C. lectularius*. In the latter, the number of autosomes is consistently 26, whereas the number of X chromosomes varies from two (X_1X_2Y) to 15 (X_1X_2Y + 13 extra Xs) in different populations and sometimes between males of one population. Autosomal bivalents show normal a sequence

of meiotic divisions, with homologous chromosomes segregating in the first round of meiosis, and sister chromatids separating in the second. This order is however reversed in sex chromosomes, and this is true even where 15 Xs exist. Sex chromosomes always undergo post-reductional meiosis, i.e. the equational separation at anaphase I and the reductional segregation at anaphase II, the sex chromosome behavior typical of the Heteroptera. Autosomal bivalents are suggested to be chiasmate, with the single chiasma being formed in every bivalent (Ueshima, 1966, 1979).

All the published cytogenetic studies on *C. lectularius* have used a standard chromosome technique. In the present study, a wild North-Western Russian population of *C. lectularius* was analyzed using the standard Schiff-Giemsa technique, $AgNO_3$-staining, fluorochrome DAPI/CMA_3-banding, and fluorescent *in situ* hybridization (FISH) with an *18S rDNA* probe. As a result, the karyotype, sex chromosome system, the distribution and nucleotide sequences of C-heterochromatin regions, chromosomal location of *18S rRNA* genes, and meiosis in males are reported here.

Material and methods

Insects

Adults and nymphs of *Cimex lectularius* were collected in 2009 from a wild population (St. Petersburg). A total of 6 males were studied. Specimens were fixed alive in 96% ethanol: glacial acetic acid (3:1) and stored in fixative at 4°C until further use.

Preparations

The gonads were dissected out and squashed in a drop of 45% acetic acid. The cover slip was removed by the dry ice method. Slides were dehydrated in fresh fixative and air dried. The preparations were first analyzed with a phase contrast microscope at 400x. The best 28 chromosome preparations were used for different staining techniques.

Standard staining

To study the number and behavior of chromosomes, the preparations were stained following the Schiff-Giemsa method developed by Grozeva, Nokkala (1996). The preparations were first subjected to hydrolysis in 1 N HCl at room temperature for 20 min, then in 1 N HCl at 60°C for 8 min, and stained in Schiff's reagent for 20 min. After rinsing thoroughly in distilled water, the preparations were additionally stained in 4% Giemsa in Sorensen's buffer, pH 6.8 for 20 min, rinsed with distilled water, air-dried, and mounted in Entellan.

$AgNO_3$ -staining

To check the localization of nucleolar organizer regions (NORs), the 1-step method with a protective colloidal developer (Howell, Black, 1980) was followed.

Fluorochrome banding

To reveal the base composition of C-heterochromatin, staining by GC-specific chromomycin A_3 (CMA_3) and AT-specific 4-6-diamidino-2-phenylindole (DAPI) were used according to Schweizer (1976), and Donlon, Magenis (1983) respectively, with some modifications. C-banding pretreatment was first carried out using 0.2 N HCl at room temperature for 30 min, followed by 7-8 min treatment in saturated Ba(OH)2 at room temperature and then an incubation in 2x SSC at 60°C for 1 h. The preparations (without Giemsa) were stained first with CMA_3 (2,5 µg/ml) for 25 min and then with DAPI (0.4 µg/ml) for 5 min. After staining, the preparations were rinsed in the McIlvaine buffer, pH 7 and mounted in an antifade medium (700 µl of glycerol, 300 µl of 10 mM McIlvaine buffer, pH 7, and 10 mg of N-propyl gallate).

Fluorescence in situ hybridization (FISH)

DNA isolation, PCR amplification, probe generation

Genomic DNA from a male of *Pyrrhocoris apterus* L., 1758 (Heteroptera, Pyrrhocoridae) was isolated using a Chelex-100 extracted method. FISH was carried out on *C. lectularius* chromosomes using an *18S rDNA* gene probe. The target *18S rRNA* gene was PCR amplified from the genomic DNA of *P. apterus* using primers: 18S_R 5'-CGATACGCGAAT GGCTCAAT-3', 18S_F 5'-ACAAGGGGCACGGACGTAATCAAC -3', and labeled by PCR with Biotin.

FISH

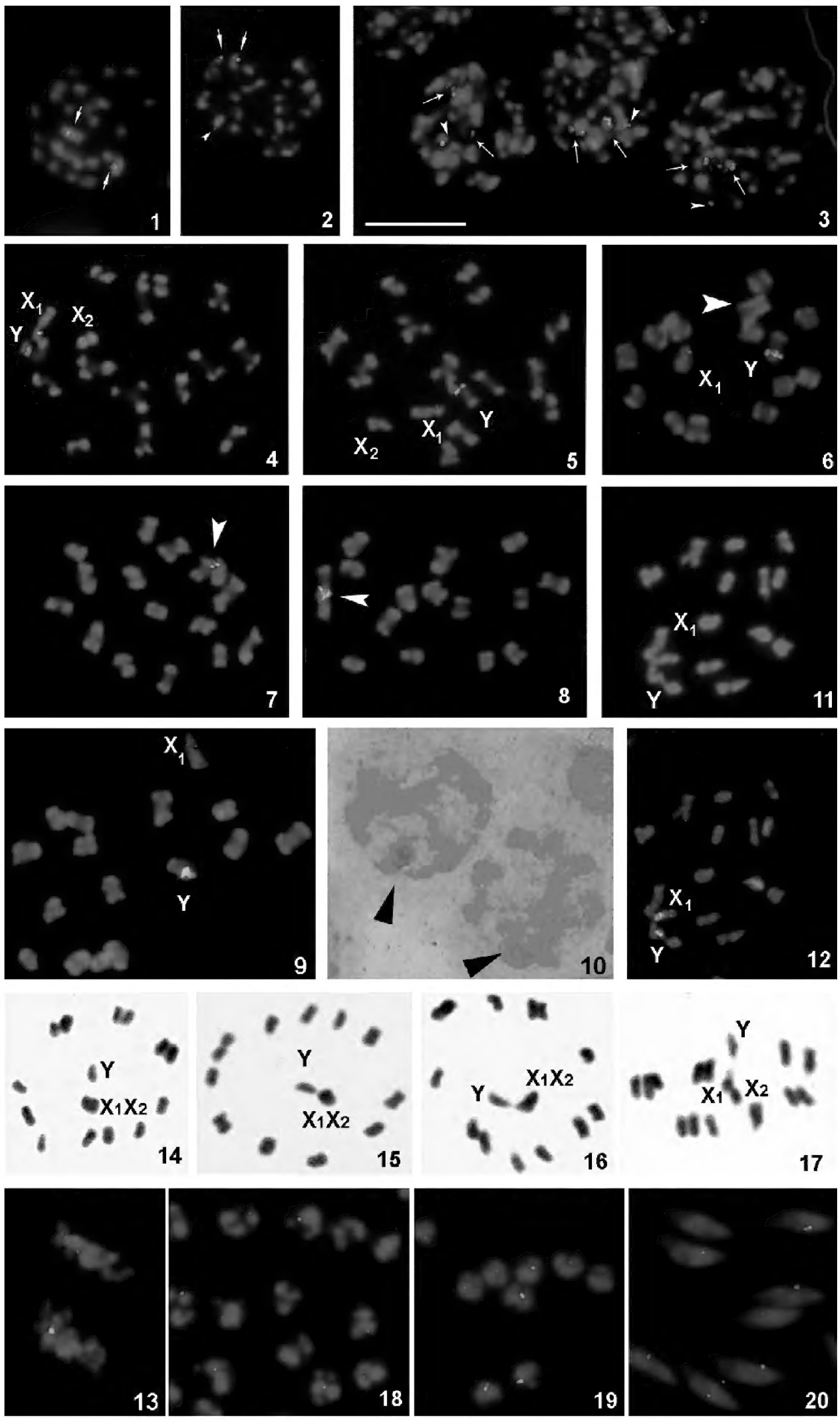
In situ hybridization was performed as described by Schwarzacher, Heslop-Harrison (2000) with modifications. Chromosome preparations were dehydrated through 70/80/96% ethanol at RT and treated with 100 µg/ml RNaseA (Sigma) for 60 min at 37°C in humid chamber; washed three times in 2x SSC (5 min each) at RT; dehydrated through 70/80/96% ethanol at RT; incubated in 5 mg/ml pepsin in 0.01 N HCl for 15 min at 37°C; washed sequentially in 1x PBS, in PBSx1/0.05M $MgCl_2$ for 5 min each, in 1% PFA in PBSx1/0.05M $MgCl_2$ for 10 min, in 1x PBS for 5 min, in PBSx1/0.05M $MgCl_2$ for 5 min at RT each; dehydrated through 70/80/96% ethanol at RT or at ice cold and finally dried. After pretreatment hybridization mixture containing about 100 ηg of labeled probe, 50% formamide, 2×SSC, 10% (w/v) dextran sulfate, 1% (w/v) Tween-20 and 10 µg salmon-sperm DNA was added on preparations. Slides were mounted using glass coverslip and rubber cement. The slides were denatured for 5 min at 75°C. Then the chromosome slides were incubated for 42–44 h at 37°C. Following hybridization, the slides were washed in 2x SSC for 3 min at 45°C, then in 50% formamide in 2x SSC for 10 min at 45°C, twice in 2x SSC (10 min each), twice in 0.2x SSC (10 min each) at 45°C, blocked in 1.5% (w/v) BSA/4x SSC/0.1% Tween-20 for 30 min at 37° in a humid chamber. *18S rRNA* gene probes were detected with 5 µg/ml Avidin-Alexa Fluor 488 (Invitrogen). The detection reaction was performed in 1.5 % BSA/ 4x SSC/ 0.1% Tween-20 for 1 h at 37°C. Slides were washed three times in 4x SSC/ 0.02% Tween-20 (10 min each) at 45° and dehydrated through 70/80/96% ethanol at RT. Chromosome preparations were mounted in an mounting-antifade (ProLong Gold antifade reagent with DAPI, Invitrogen) and covered with a glass coverslip.

Microscopy and imaging

Chromosome preparations were analyzed under a Leica DM 4000B microscope (Leica Microsystems Wetzlar GmbH, Germany) with a 100x objective. Fluorescence images were taken with a Leica DFC 350 FX camera using Leica Application Suite 2.8.1 software with an Image Overlay module. The preparations were stored partly at the Institute of Biodiversity and Ecosystem Research, BAS in Sofia and partly at the Zoological Institute, RAS in St Petersburg.

Results

We analyzed meiosis in males of *C. lectularius* using the standard chromosome technique, $AgNO_3$-staining, fluorochrome DAPI/CMA_3 staining and *18S rDNA* FISH technique. Based primarily upon the behavior of chromosomes in the consecutive stages of meiosis, the chromosome complement in males was expressed as a formula 2n = 29 (26A + X_1X_2Y). Although females were not studied, sex chromosome mechanism was referred to a X_1X_2Y system (rather than XY_1Y_2) due to the Ueshima's (1966) observations of several populations of *C. lectularius* in which females showed 4Xs ($X_1X_2X_3X_4$) while males 2Xs


1
2
3
X1
X2
Y
4
X2
X1
Y
5
X1
Y
6
7
8
X1
Y
11
X1
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X1
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12
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X1X2
14
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X1X2
15
Y
X1X2
16
Y
X1
X2
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20

(X_1X_2) and Y respectively (see Discussion). The analysis of the chromosomes after FISH with an *18S rDNA* probe as well after CMA_3-staining evidenced the presence of the *18S rDNA* loci on the X_1 and Y chromosomes. This marker allowed the precise identification of the X_1 and Y chromosomes to be made in mitotic cells (Figs 1-3) and at different stages of meiosis (Figs 4-9). The location of the NORs on the sex chromosomes was confirmed by the $AgNO_3$-staining (Fig. 10)

The mitotic chromosomes gradually decrease in size and lack visible constrictions since they are holokinetic. Consequently, neither homologous chromosomes nor sex chromosomes are possible to identify in the routinely stained preparations (not shown). The X_1 and Y chromosomes can be however easily detected both in metaphase and prometaphase cells after FISH with the *18S DNA* probe (Figs 1-3). The majority of the mitotic cells show two chromosomes, most likely X_1 and Y, with clusters of *rRNA* genes (Figs 1, 2); however, hybridization signals are well over two in some prometaphase cells (Fig. 3).

Among the meiotic stages, those referred to the prophase and early metaphase I (MI) are of chief interest for characterisation of male meiosis in *C. lectularius*. During prophase the chromosomes gradually condense from leptotene to pachytene, and no diplotene or diakinesis stages are observed. In the course of condensation, the homologues of bivalents open out, while remaining connected with each other, at least, at one site by tenacious thread-like structures (Figs 4, 5). By the metaphase I (MI), bivalents are completely condensed and consist of parallel-aligned chromosomes with occasionally one or both ends diverging, and no signs of the existence of chiasmata are seen during this stage (Figs 6-9). Taken together these observations are indicative of achiasmate meiosis, the connecting threads between homologues representing the collochores previously described in several Miridae species (Nokkala, Nokkala, 1986) and recently also in *C. emarginatus* Simov, Ivanova, Schunger, 2006 (as *Cimex* sp. in Grozeva, Nokkala, 2002).

At MI, 13 autosomal bivalents and 3 sex chromosome univalents are observed (Figs 7-9). MI plates are nonradial with X_1, X_2, and Y chromosomes located among the bivalents. The sex chromosomes are usually recognized at this stage because they are composed of two chromatids (contrary to 4 in autosomal bivalents). The autosomal bivalents are of gradually decreasing size with neither very large nor very small entities as do the autosomes at the mitotic metaphases. The sex chromosomes are similar in size to the larger half-bivalents, the X_1 being clearly longer than the X_2 while of similar size with the Y. Sex chromosomes, X_1 and Y at least, tend to be located close to each other or even connected by ends probably due to the presence of

Figs 1-20. Different stages of male meiosis in *C. lectularius* after FISH with an *18S rDNA* probe (**1-9, 13, 18-20**), $AgNO_3$ (**10**), DAPI (**11**), CMA_3 (**12**), and Schiff-Giemsa (**14-17**). **1-3** - Spermatogonial metaphases. Arrows show X_1 and Y with signals, arrowheads point to additional signals in some plates; **4-6** - Prometaphase I. X_1 and Y with signals lie together (**4**) or separately (**5, 6**); **7-9** - Metaphase I. X_1 and Y with signals lie together or separately. Autosomal bivalents are condensed and consist of parallel-aligned chromosomes; **10** - Diffuse stage. Nucleolar proteins are localized on the sex chromatin body; **11-12** - A metaphase I plate. X_1 and Y with CMA_3 -positive signals (**12**); **13** - Anaphase I. There are signals in both daughter cells; **14-17** - Metaphase II. Radial plates with sex chromosomes placed inside the ring formed by autosomes; **18-20** - Consecutive stages of sperm formation. Every sperm has a signal. Bar = 10μm.

telomere heterochromatin (Figs 7, 8). Among the 26 MI cells examined, only two showed X_1 and Y chromosomes lying separately (Fig. 9).

In two MI nuclei after DAPI and CMA_3-staining respectively, the autosomal bivalents and one of the Xs (X_2) display a fairly homogenous CMA_3/DAPI staining, however at least two bivalents (arrowed) show CMA_3/DAPI positive, AT/GC rich heterochromatic regions on the telomeres of every homologue (Figs 11, 12). The other X (X_1) and the Y chromosome exhibit several CMA_3-bright bands, two-three on the X_1 and one-two on the Y, indicating they are GC-rich. FISH experiments are in general agreement with the results of CMA_3-staining, i.e. the *18S rDNA* loci are co-localized with some of the CMA_3-bands suggesting that these CMA_3-positive sites represent *rDNA* cistrons. X_1 and Y chromosomes each show hybridization signals indicating clusters of *18S rRNA* genes are located near the end of X_1 and near the end of Y chromosomes respectively (Figs 7-9). Noteworthy is a size polymorphism for the *18S rDNA* clusters on X_1 and Y, which is occasionally observed even within the same male (Figs 6-9).

The daughter metaphase II (MII) cells each contain 16 chromosomes, including 13 autosomes, 2Xs, and the Y (Figs 14-17), indicative of the reductional segregation of autosomes and equational separation of sex chromosomes at anaphase I (AI) (Fig. 13). At MII, sex chromosomes undergo a characteristic "touch-and-go" pairing, with 2Xs lying on one side of the equatorial plate and the Y on the other. In contrast to MI, metaphase II (MII) plates are clearly radial, and sex chromosomes appear here as a pseudotrivalent located inside a ring formed by autosomes.

At anaphase II (AII), autosomes divide equationally, but sex chromosomes undergo a reductional division, i.e. both X chromosomes moved to one pole and the Y to the other (the so-called sex chromosome post-reduction) as evidenced by the only *18S rDNA*/FISH signal in every daughter telophase II (TII) plate (Fig. 18) as well as in every spermatid and every sperm (Figs 19, 20).

Discussion

The common bed bug, *Cimex lectularius*, is one of the most widely recognized insects all over the world. Slack (1938) was the first to study chromosome cytology of *C. lectularius*. Shortly afterwards Darlington (1939) and then Ueshima (1966, 1967, 1979) studied and discussed the unique aspects of male meiosis in this species. Based on the observations of these authors, *C. lectularius* males have the standard complement of $2n = 26 + X_1X_2Y$, the X chromosomes varying in number from two (X_1X_2Y) to 15 (X_1X_2Y + 13 extra Xs) in different populations while occasionally between males of the same population and even between cells of the same male. For example, Ueshima (1966) has investigated males and females in six populations originated from USA (Berkeley, California; Columbus, Ohio), Mexico (Monterey; La Piedad), Japan (Nagasaki), France (Durtal), Egypt (Cairo), and Czech Republic (Moravia) respectively. The number of sex chromosomes was shown to be stable within every population (2Xs in Berkeley, La Piedad, Nagasaki, and Durtal; 6Xs in Cairo and Moravia) except for the Ohio population, in which males had either 7Xs or 9Xs. Notice that the transmission of additional sex chromosomes throughout meiosis was, except in a very few cases, quite regular, and they seemed not to be important for sex determination.

Multiple (above two) X chromosomes have been described in both natural populations and laboratory stocks of *C. lectularius*. According

to Darlington (1939) the average number of Xs is higher in wild populations than in laboratory cultures, however a closer look at the presently available data on this species is called for.

The most common sex chromosome mechanism in the Heteroptera is XX/XY system, and the multiple sex chromosome systems are suggested to have originated from the original XY system (Ueshima, 1979). In the Cimicidae, a total of 45 species have been hitherto cytologically studied and both putative ancestral XY and a great number of derived multiple systems, among which X_1X_2Y clearly prevails, were found (Ueshima, 1966, 1979 and references therein; Manna, 1984; Grozeva, Nokkala, 2002; Poggio et al., 2009). This is also true for the genus *Cimex* in which as many as 14 of the 17 described species have been studied (Ueshima, 1963, 1966, 1967; Grozeva, Nokkala, 2002; for other references see Ueshima, 1979). The origin of multiple systems in the Heteroptera is usually ascribed to simple transverse dissociation (fission) of the original X chromosome (Schrader, 1947; Ueshima, 1966, 1979), the process which is facilitated by the holokinetic nature of the bugs' chromosomes. The distinguishing features of multiple sex chromosome systems formed by dissociation are that the newly originated sex chromosomes are smaller than the original ones, and there is no accompanying change in the number of autosomes in a derived complement compared to that with XY sex chromosome system. Ueshima (1966) has discovered XY, X_1X_2Y, and $X_1X_2X_3X_4Y$ systems in 7 species of the *Cimex pilosellus* (Horváth, 1910) complex and argued for the dissociation hypothesis on the basis that as the number of X chromosomes increases, their size decreases. However this problem clearly calls for further investigation using modern cytological techniques. It is noteworthy that the application of C-banding to study the chromosomes of several Triatominae (Reduviidae) species, led Panzera et al. (2010) to the conclusion that chromosomal rearrangements other than dissociations might have been involved in the formation of the multiple sex chromosome systems in the Heteroptera.

As is typical in the Heteroptera, chromosomes of *C. lectularius* are holokinetic, i.e. without localized centromere. In males studied herein meiosis follows a standard for the Heteroptera pattern as previously described in other populations of this species (Slack, 1939b; Darlington, 1939; Ueshima, 1966, 1979). The sex chromosomes behave as univalents during the first round of meiosis and undergo equational separation at anaphase I. At metaphase II, the Xs and Y appear associated end-to-end to form a pseudotrivalent, which is located inside the ring of autosomes. During anaphase II, Xs and Y chromosomes undergo reductional division segregating to opposite poles. This reversed order of sex chromosome behavior during meiotic divisions is characteristic of the Heteroptera, referred to as inverted meiosis or sex chromosome post-reduction (Hughes-Schrader, Schrader, 1961).

At present, the cytogenetic studies of the Heteroptera are mainly focused on C-heterochromatin and nucleolus organizer regions (NORs), whereas molecular cytogenetic techniques such as immunofluorescence ones and different modifications of FISH (fluorescent *in situ* hybridization), including chromosome painting, BAC-FISH technique, and GISH/FISH mapping of genes, are not yet used in the Heteroptera. By now, some of these techniques have been applied to some economically important holokinetic insects (aphids *Acyrthosiphon pisum* (Harris, 1776); cabbage moth *Mamestra brassica* (Linnaeus, 1758); silkworm *Bombyx mori* (Linnaeus, 1758)) and have provided useful insight into

the understanding of their genome constitution (Mandrioli et al. 2003; Yoshido et al., 2005; Mandrioli, Borsatti, 2007; Marec et al., 2010).

The base-specific fluorochromes DAPI and CMA_3 are currently widely applied in cytogenetic studies of the Heteroptera to get information about the distribution of the AT and GC repeats along the chromosomes. In most heteropteran species the only pair of NOR-bearing chromosomes has been detected by different techniques such as fluorochrome banding, Ag-NOR staining or occasionally FISH with an *rDNA* probe. The location of NORs is however variable. In different species, the NORs are revealed on autosomes or on sex chromosomes (see reviews by Papeschi and Bressa, 2006a, b) but occasionally they occur on both sex chromosomes and autosomes of a species (Morielle-Souza, Azeredo-Oliveira, 2007; Bressa et al., 2008).

In our study of *C. lectularius*, the two CMA_3-positive GC-rich bands were discovered at the telomeres of sex chromosomes (X_1 and Y). NORs are known to be largely GC-rich in the Heteroptera (Grozeva et al., 2004; Papeschi, Bressa, 2006a, b; Kuznetsova et al., 2007), and CMA_3^+/$DAPI^-$- regions of *C. lectularius* were therefore interpreted as the sites of NORs, and FISH with the *18S rDNA* probe confirmed this assessment. As expected, FISH/rDNA signals were observed in every spermatid, confirming thus the location of NORs on one of the X chromosomes (X_1) and on the Y chromosome, which underwent a reductional division at anaphase II.

Ueshima (1966, 1979) has argued for chiasmate meiosis in *C. lectularius* with one chiasma being formed in every bivalent. However, our study did not support this generalization suggesting the occurrence of achiasmate meiosis in *C. lectularius*, and this pattern seems to be characteristic of the family Cimicidae as a whole (Grozeva, Nokkala, 2002; Poggio et al., 2009). Strong support for this suggestion is known to come from the absence of diplotene and diakinesis stages (Nokkala, Nokkala, 1986) and such is the case in *C. lectularius*. According to our observations, males of *C. lectularius* follow achiasmate meiosis of the specific collochore type. In this meiosis there are no chiasmata; after synapsis, the opening-out of bivalents takes place, and tenacious threads, the so-called collochores, are formed to hold homologous chromosomes together in the absence of chiasmata. The collochore meiosis was first described in *Drosophila melanogaster* Meigen, 1830 (Cooper, 1964), thereafter in four Miridae species (Nokkala, Nokkala, 1986) and quite recently in *Cimex emarginatus* (as *Cimex* sp. in Grozeva, Nokkala, 2002) and some other Cimicidae species (Poggio et al., 2009). One further type of achiasmate meiosis known in the Heteroptera is the alignment type. In this meiosis, homologous chromosomes are held together along all their length during prophase, this alignment remaining unchanged till the beginning of anaphase I (Nokkala, Nokkala, 1983, 1984; Nokkala, Grozeva, 2000; Grozeva et al., 2008). Furthermore, a pattern intermediate between collochore and alignment meioses has been quite recently described in *Arachnocoris trinitatus* Bergroth, 1916 from the Nabidae (Kuznetsova et al., 2007; Kuznetsova, Grozeva, 2008), the cimicomorphan family with primarily meiosis of the alignment type (Nokkala, Nokkala, 1984; Kuznetsova, Maryańska-Nadachowska, 2000; Kuznetsova et al., 2004).

The form of metaphase I and metaphase II plates in *C. lectularius* is noteworthy, and this is the pattern which seems to be species-specific in the Heteroptera (Ueshima, 1979). In this species, the metaphase I plates were found to be nonradial but metaphase II plates radial.

It is interesting to note in this connection that in another Cimicidae species, *Psiticimex uritui* Lent, Abalos, 1946, both MI and MII plates seem to be radial (Poggio et al., 2009: Figs 2b, c).

Conclusions

The results of the present study support the following assertions: (1) *Cimex lectularius* displays holokinetic chromosomes; (2) male karyotype includes 2n = 26 + X_1X_2Y; (3) *18S rRNA* genes are located on the X_1 and Y chromosomes; (4) males have achiasmatic meiosis of the collochore type; (5) sex chromosomes undergo post-reductional meiosis, i.e. the equational separation at AI and the reductional segregation at AII; (6) MI plates are radial and MII plates are nonradial.

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References

Bressa M.J., Franco M.J., Toscani M.A., Papeschi A.G. 2008. Heterochromatin heteromorphism in *Holhymenia rubiginosa* (Heteroptera, Coreidae) // *European J. Entomol.* 105: 65-72.

Cooper K.W. 1964. Meiotic conjunctive elements not involving chiasmata // *Proc. Nat. Acad. Sci. USA* 52: 1248-1255.

Darlington C.D. 1939. The genetical and mechanical properties of the sex chromosomes. V *Cimex* and the Heteroptera // *J. Genet.* 39: 101–137.

Donlon T.A., Magenis R.E. 1983. Methyl green is a substitute for distamycin A in the formation of distamycin A/DAPI C-bands // *Human Genetics*. 65: 144-146.

Grozeva S., Kuznetsova V.G., Nokkala S. 2004. Patterns of chromosome banding in four nabid species (Heteroptera, Cimicomorpha, Nabidae) with high chromosome number karyotypes // *Hereditas*. 140(2): 99-104.

Grozeva S., Nokkala S. 1996. Chromosomes and their meiotic behavior in two families of the primitive infraorder Dipsocoromorpha (Heteroptera) // *Hereditas*. 125: 31-36.

Grozeva S., Nokkala S. 2002. Achiasmatic male meiosis in *Cimex* sp. (Heteroptera, Cimicidae) // *Caryologia*. 55 (3): 189-192.

Grozeva S., Nokkala S., Simov S. 2008. Achiasmatic meiosis in two *Micronecta* species (Heteroptera, Nepomorpha, Micronectidae) // *Comp. Cytogenet.* 2(1): 73-78.

Howell W.M., Black D.A. 1980. Controlled silver staining of nucleolus organizer region with a protective colloidal developer: a 1-step method // *Experientia*. 36: 1014-1015.

Hughes-Schrader S., Schrader F. 1961. The kinetochore of the Hemiptera // *Chromosoma*. 12: 327-350.

Kuznetsova V.G., Grozeva S., Nokkala S. 2004. New cytogenetic data on Nabidae (Heteroptera: Cimicomorpha), with a discussion of karyotype variation and meiotic patterns, and their taxonomic significance // *European J. Entomol.* 101: 205-210.

Kuznetsova V.G., Grozeva S., Sewlal J.N., Nokkala S. 2007. Cytogenetic characterization of the endemic of Trinidad, *Arachnocoris trinitatus* Bergroth: the first data for the tribe Arachnocorini (Heteroptera: Cimicomorpha: Nabidae) // *Folia Biol.* (Kraków). 55(1-2): 17-26.

Kuznetsova V.G., Grozeva S. 2008. Cytogenetic characters of *Arachnocoris trinitatus* Bergroth, 1916 (Insecta, Heteroptera: Nabidae) from nests of the spider *Coryssocnemis simla* Huber, 2000 (Araneae: Pholcidae) // *Comp. Cytogenet.* 2(2): 139-142.

Kuznetsova V.G., Maryańska-Nadachowska A. 2000. Autosomal polyploidy and male meiotic pattern in the bug family Nabidae (Heteroptera) // *J. Zool. Syst. Evol. Res.* 38(2): 87-94.

Morielle-Souza A., Azeredo-Oliveira M.T.V. 2007. Differential characterization of holocentric

chromosomes in triatomines (Heteroptera, Triatominae) using different staining techniques and fluorescent *in situ* hybridization // *Genet. Mol. Res.* 6: 713-720.

Mandrioli M., Manicardi G.C., Marec F. 2003. Cytogenetic and molecular characterization of the MBSAT1 satellite DNA in holokinetic chromosomes of the cabbage moth, *Mamestra brassicae* (Lepidoptera) // *Chromosome Res.* 11: 51-56.

Mandrioli M., Borsatti F. 2007. Analysis of heterochromatic epigenetic markers in the holocentric chromosomes of the aphid *Acyrthosiphon pisum* // *Chromosome Res.* 15: 1015-1022.

Manna G.K. 1984. Chromosomes in evolution in Heteroptera, (pp. 189-225) // Sharma A.K., Sharma A. (Eds). *Chromosomes in Evolution of Eukaryotyc groups. 2*. Boca Raton, Florida. 269 p.

Marec F., Sahara K., Traut W. 2010. Rise and fall of the W chromosome in Lepidoptera, (pp. 49-63) // Goldsmith, M.R., Marec, F. (Eds) *Molecular Biology and Genetics of the Lepidoptera*. Boca Raton, Florida. 362 p.

Morielle-Souza A., Azeredo-Oliveira M.T.V. 2007. Differential characterization of holokinetic chromosomes in triatomines (Heteroptera, Triatominae) using different staining techniques and fluorescent *in situ* hybridization // *Genet. Mol. Res.* 6(3): 713-720.

Nokkala S., Grozeva S. 2000. Male meiosis of achiasmatic type in *Myrmedobia coleoptrata* (Fn.) (Heteroptera, Microphysidae) // *Caryologia*. 53(1): 5-8.

Nokkala S., Nokkala C. 1983. Achiasmatic male meiosis in two species of *Saldula* (Saldidae, Hemiptera) // *Hereditas*. 99: 131-134.

Nokkala S., Nokkala C. 1984. Achiasmatic male meiosis in the Heteropteran genus *Nabis* (Nabidae, Hemiptera) // *Hereditas*. 101: 31-35.

Nokkala S., Nokkala C. 1986. Achiasmatic male meiosis of collochore type in the heteropteran family Miridae // *Hereditas*. 105: 193-197.

Panzera F., Perez R., Panzera Y., Ferrandis I., Ferreiro M.J., Calleros L. 2010. Cytogenetics and Genome Evolution in the Subfamily Triatominae (Hemiptera, Reduviidae) // *Cytogen. Genome Res.* 128(1-3): 77-87.

Papeschi A.G., Bressa M.J. 2006a. Classical and molecular cytogenetics in Heteroptera // *Res. Adv. Entomol.* 1: 1-9.

Papeschi A.G., Bressa M.J. 2006b. Evolutionary cytogenetics in Heteroptera // *J. Biol. Res.* 5: 3-21.

Poggio M.G., Bressa M.J., Papeschi A.G., di Iorio O., Turienzo P. 2009. Insects found in birds' nests from Argentina: cytogenetic studies in Cimicidae (Hemiptera) and its taxonomical and phylogenetic implications // *Zootaxa*. 2315: 39-46.

Schrader F. 1947. The role of kinetochore in the chromosome evolution of the Heteroptera and Homoptera // *Evolution*. 14: 498-508.

Schuh R.T., Slater J.A. 1995. True Bugs of the World (Hemiptera: Heteroptera): Classification and Natural History. Ithaca, New York. 336 p.

Schwarzacher T., Heslop-Harrison P. 2000. Practical *in situ* Hybridization. BIOS. Scientific Publishers Ltd. Oxford, UK. 338 p.

Schweizer D. 1976. Reverse fluorescent chromosome banding with chromomycin and DAPI // *Chromosoma*. 58: 307-324.

Simov N., Ivanova T., Schunger I. 2006. Bat-parasitic *Cimex* species (Hemiptera: Cimicidae) on the Balkan Peninsula, with zoogeographical remarks on *Cimex lectularius* Linnaeus // *Zootaxa*. 1190: 59-68.

Slack H.D. 1938. Chromosome Numbers in *Cimex* // *Nature*. 142: 358.

Slack H.D. 1939a. Chromosomes of *Cimex* // *Nature*. 143: 78.

Slack H.D. 1939b. Structural hybridity in *Cimex* L. // *Chromosoma*. 1: 104-118.

Ueshima N. 1963. Chromosome behavior of the *Cimex pilosellus* complex (Cimicidae: Hemiptera) // *Chromosoma*. 14: 511-521.

Ueshima N. 1966. Cytology and cytogenetics (pp. 183-237) // Usinger R.L. (Ed.), *Monograph of Cimicidae*. New York. 585 p.

Ueshima N. 1967. Supernumerary chromosomes in the human bed bug, *Cimex lectularius* L. (Cimicidae: Hemiptera) // *Chromosoma*. 20: 311-331.

Ueshima N. 1979. Hemiptera II: Heteroptera. *Animal Cytogenetics 3: Insecta 6*. Berlin, Stuttgart. 113 p.

Yoshido A., Marec F., Sahara K. 2005. Resolution of sex chromosome constitution by genomic *in situ* hybridization and fluorescence *in situ* hybridization with $(TTAGG)_n$ telomeric probe in some species of Lepidoptera // *Chromosoma*. 114: 193-202.

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